Accelerated Photo-Induced Hydrophilicity Promotes Osseointegration: An Animal Study

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ABSTRACT

Background: In the previous in vitro study, fluoride-modified, anodized porous titanium was proven to have enhanced its photo-induced hydrophilicity, which induced the hyperactivation of initial cell response.

Purpose: The purpose of the present study was to investigate in vivo bone apposition during the early stages of osseointegration in rabbit tibiae.

Materials and Methods: Anodized porous titanium implants (TiU, TiUnite®, Nobel Biocare AB, Göteborg, Sweden) were modified with 0.175 wt% ammonium hydrogen fluoride solution (NH_4F-HF_2). Twenty-four hours prior to the experiments, the surface-modified implants were ultraviolet-irradiated (modTiU). Blinded and unpackaged TiU implants were used as controls. Thereafter, the implants were placed in the rabbit tibial metaphyses and histomorphometrically analyzed at 2 and 6 weeks after insertion.

Results: ModTiU demonstrated a significantly greater degree of bone-to-metal contact than TiU after 2 and 6 weeks of healing.

Conclusion: The results proved that the enhanced photo-induced hydrophilicity of the NH_4F-HF_2-modified anodized implants promoted bone apposition during the early stages of osseointegration.

KEY WORDS: anatase TiO_2, enhanced bone apposition, fluoride, osseointegration, photo-induced hydrophilicity

INTRODUCTION

The implant surface finish, that is, the surface property, has been recognized as an important factor of successful osseointegration.[1] Ever since this factor was proposed, surface topography has focused on promoting early and secure bone formation around dental implants.[2,3] Consequently, moderately increased surface roughness with the Sa value of around 1.5 µm is known to provide advantageous surface properties for favorable bone response.[4,5] Different implant manufacturers have attempted to obtain a so-called "moderately roughened" surface by particle blasting, acid etching, or anodizing. These modifications have boosted the success rate, especially in patients with poor bone quality sites[6] and have also reduced length of the healing period.[7,8]

In recent years, chemically modified surfaces have been proven to enhance the speed and firmness of osseointegration. Several studies have reported that chemically cleaned microstructured surfaces enhanced the bone apposition during the early stages of bone regeneration.[9,10] These modifications increase the hydrophilic property of titanium implant surfaces and enhance the initial adsorption of extracellular matrix proteins.

It has been discovered and reported that anodized porous titanium dioxide (TiO_2) implants acquire photo-induced hydrophilicity when irradiated with ultraviolet (UV) light.[11] The water contact angle for the ordinary anodized porous TiO_2 implants is 44°, whereas it dramatically decreased to 11° after 24 hours of UV irradiation, which indicates that anodized porous TiO_2

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implants have inherent photo-induced hydrophilicity. However, no significant enhancement of bone regeneration around the UV-irradiated anodized porous TiO_2 implants after 4 weeks of healing in the rabbit tibiae could be seen. It was speculated that this might be a result of the insufficient level of surface hydrophilicity. Therefore, in the previous study, the hydrophilicity of the implants was improved by fluoride modification.[12] In brief, modification with 0.175% ammonium hydrogen fluoride (NH_4F-HF_2) enhanced the hydrophilic property of the anodized porous TiO_2 and improved the initial cell response. After a short culturing time, the mesenchymal cells showed a significant increase in cell attachment. The corresponding cell morphology was well flattened, with numerous lamellipodial extensions. In addition, the proliferative activity was significantly accelerated after 24 hours.

With the in vitro results being acknowledged, it was assumed in this study that further improvement of the photo-induced hydrophilic surface of the anodized porous TiO_2 implant would dramatically enhance the initial stages of bone regeneration, and, therefore, the histological transitions occurring during the osseointegration cascade in vivo were investigated.

MATERIALS AND METHODS

Confirmation of Hydrophilicity

The effect of NH_4F-HF_2 treatment on the hydrophilicity of the implant was confirmed by assessing the static contact angles with a (FACE) contact angle analyzer (Kyowa Interface Science Co. Ltd., Asaka, Japan) using the sessile drop technique.[13] Ten disks (ø11.5 × 2 mm) subjected to the same surface treatment as the commercial TiUnite implants were specially fabricated and supplied by Nobel Biocare AB, Göteborg, Sweden. All specimens were ultrasonically degreased in trichloroethylene for 15 minutes, then soaked in 95% ethanol for 15 minutes and in distilled water for 15 minutes three times. Half of the disks were immersed in 0.175% NH_4F-HF_2 solution for 5 minutes, which was the highest hydrophilic concentration used in the previous study,[12] and irradiated with UV at a peak wavelength of 352 nm for 24 hours in advance (DmodTiU). The other half were neither treated with NH_4F-HF_2 solution nor irradiated with UV (DTiU). The measurements were carried out at room temperature in air with distilled water as the probe liquid. Liquid droplets (8 μL) were deposited onto the sample surface at a rate of 8 μL/s.

Implant Preparation

Thirty anodized porous TiO_2 implants (3.75 × 7 mm) were used in this experiment. Half of the implants were immersed in 0.175% NH_4F-HF_2 solution for 5 minutes. Thereafter, the specimens were ultrasonically degreased in trichloroethylene for 15 minutes, then soaked three times in 95% ethanol for 15 minutes and in distilled water for 15 minutes. Finally, the specimens were irradiated with UV for 24 hours prior to the experiment (modTiU). The other half were kept in their sterile packages and taken out shortly before the experiment (TiU). The surface roughness and morphology of three implants of each type were analyzed by using a three-dimensional-laser scanning microscope (VK-8700, Keyence, Osaka, Japan). The centerline average roughness (Ra) values for the upper flank face of the first thread were determined by averaging the values of five random areas per implant (a total of 15 areas for each group).

In Vitro Statistical Analyses

The statistical analyses for the in vitro study were performed by using the KaleidaGraph software (Synergy Software, Essex Junction, VT, USA). The mean and standard deviation values for the in vitro parameters were calculated. The average values were compared by one-way analysis of variance, followed by a post hoc Tukey–Kramer test, with the value of statistical significance set at 0.05.

Animal Experiments

Twelve adult female Japanese White rabbits (average body weight: 4.0 kg; age: 7–9 months) were included in the animal experiment. Animal care and experimental procedures were performed in accordance with the Guidelines for Animal Experimentation of Nagasaki University, with the approval of the Ethics Committee for Animal Research.

The animals were anesthetized with intramuscular injections of ketamin (0.5 mg/kg) and xylazine (0.25 mg/kg). The skin and fascial–periosteal layers were opened and closed separately. Rotary drill speeds not exceeding 2,000 rpm and saline cooling were used during all the surgical drilling sequences. Each rabbit received a modTiU and a TiU unicortically in the right

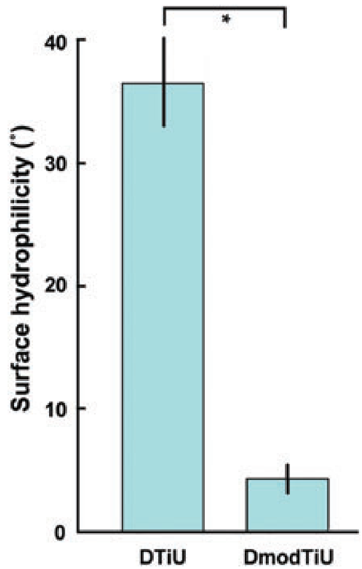

Figure 1 Water contact angle of DTiU and DmodTiU (* $p < .05$).

and left proximal tibial metaphyses. Both types of implants were evenly distributed in the left and right tibiae. After 2 and 6 weeks of healing, six rabbits per healing period were sacrificed by intravenous injections of pentobarbital.

The implants were removed en bloc and immersed in a fixative for subsequent histological investigations, and one central section per implant was prepared. The sections were ground to a final thickness of about 40 µm and stained with toluidine blue. The bone-to-metal contact (BMC) values were analyzed with respect to the three best consecutive threads, which corresponded to the cortical contact area for each specimen. The statistical calculations were also performed with the KaleidaGraph software using the Wilcoxon signed rank test ($p = .05$). In order to observe whether there is a significant enhancement of bone apposition over time, analysis was also performed within each group at 2 and 6 weeks.

RESULTS

Photo-Induced Hydrophilicity

The mean water contact angle and standard deviation (SD) of the DTiU and the DmodTiU were 36.6° (3.28) and 4.4° (0.93), respectively (Figure 1). The NH_4F-HF_2 treatment and subsequent UV irradiation (DmodTiU) significantly enhanced the surface hydrophilicity.

Surface Characteristics

The surface roughness values and three-dimensional images of TiU and modTiU are shown in Figure 2, A and B. The mean Ra values (SD) for TiU and modTiU were 1.84 (0.23) and 1.52 (0.26), respectively. There was a significant difference between the two groups, indicating that NH_4F-HF_2 treatment smoothened the surface imperceptibly.

Animal Experiment

The healings after surgery progressed uneventfully, and there were no clinical signs of infection. At the time of sacrifice, all implants were already immobilized.

Because of the early observation setup, scattered bone formation and newly formed trabeculae with deeply stained mineralized tissue were evident in both groups after 2 weeks of healing. The thin but lengthwise spread of bone contacting the implant was observed in both the TiU and the modTiU groups. It was particularly interesting that the bone was more continuous in its apposition along the implant surface of modTiU than of TiU, as shown in Figure 3A.

After 6 weeks of healing, the scattered bone around the implant disappeared, and new bone formed

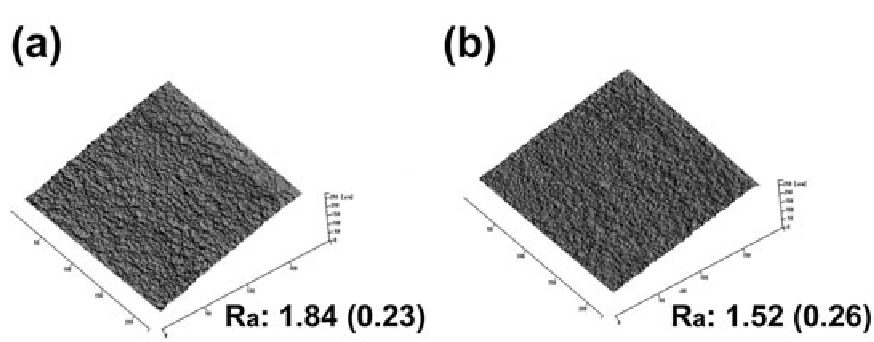

Figure 2 Laser three-dimensional microscopic images of (A) TiU with an average Ra value of 1.84 (0.23) and (B) modTiU with an average Ra value of 1.52 (0.26). (Ra = average roughness.)

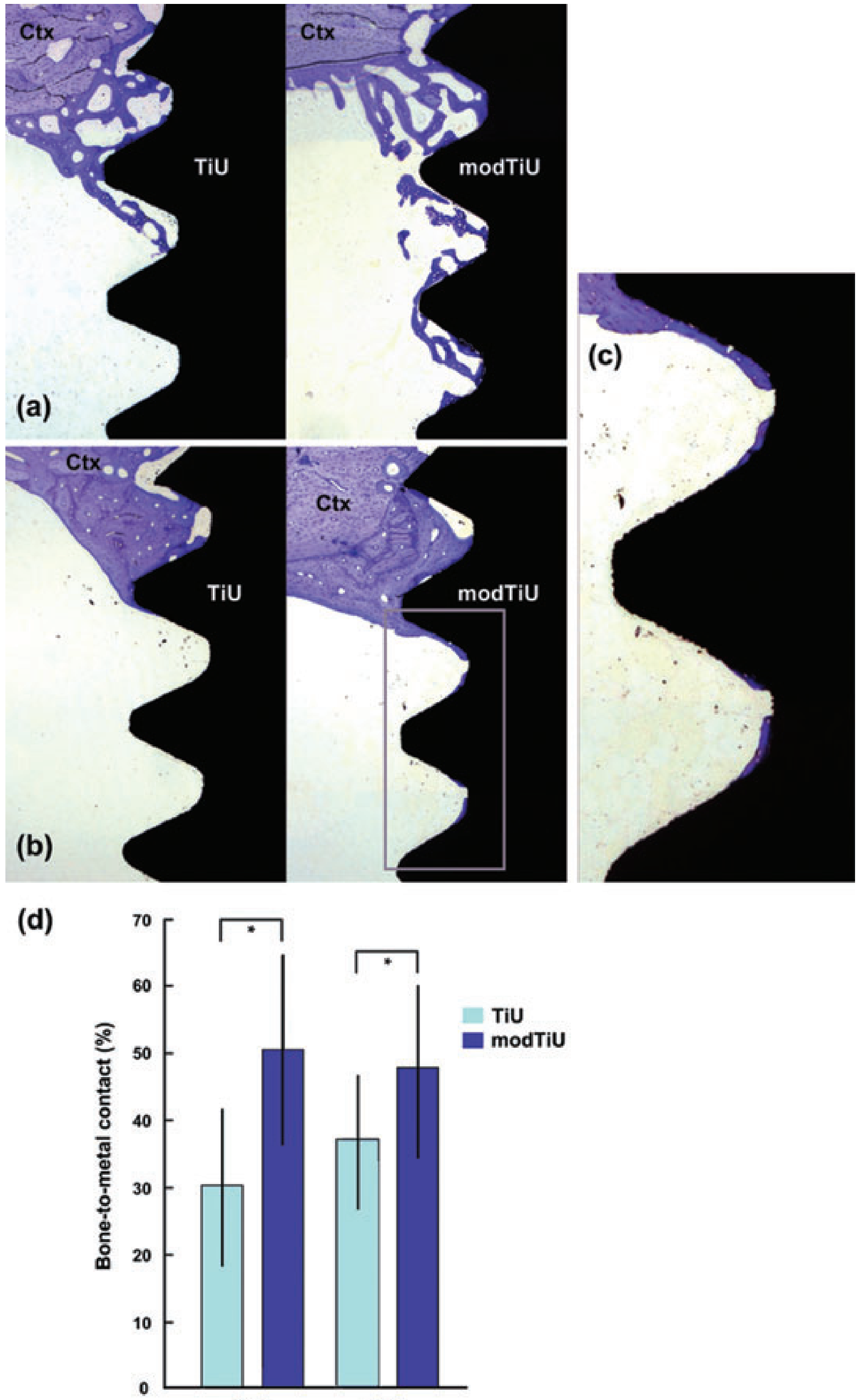

Figure 3 Light micrographs (toluidine blue staining; original magnification ×20) of TiU and modTiU after (A) 2 weeks and (B) 6 weeks of healing. Ctx: cortex (C) A more detailed micrograph showing new bone contacting the modTiU implant across the marrow region. (D) Bone-to-metal contact for TiU and modTiU after 2 and 6 weeks of healing (*$p < .05$).

osteoconductively from the cortical bone (see Figure 3B). The newly formed bone became more woven and dense, rather than trabecular in appearance, with the contraction of the bone lacuna. However, it was still possible to distinguish between the original bone and the newly formed bone because of the interrupted lamellae. Although the scattered bone disappeared in both groups, the new bone contacting the implant surface exhibited different states. In the modTiU group, bone contacted the implant surface continuously as shown in the magnified Figure 3C. On the other hand, in the TiU group, bone contacted the implant surface adjacent to the cortical area, but less contact could be observed in the marrow area.

The histomorphometric results corresponded to the histological findings (see Figure 3D). The mean BMC (SD) for TiU and modTiU at 2 weeks were 30.1% (12.1) and 50.3% (16.3), respectively. The modTiU group showed statistically significant higher BMC at 2 weeks ($p = .0036$). At 6 weeks, the BMC (SD) of modTiU was 47.6% (13.3), again, significantly higher than that of TiU, which was 37.1% (10.7) ($p = .0042$). Although the BMC decreased, there was no statistical difference between 2 and 6 weeks for the modTiU group. For the TiU group, the BMC increased, and there was a statistical difference ($p = .03418$).

DISCUSSION

Photo-induced hydrophilicity was originally discovered by Wang and colleagues,[14] who demonstrated that TiO_2 polycrystalline film made from anatase sol exhibited a dramatic decrease in the water contact angle from $72° \pm 1°$ to $0° \pm 1°$ after UV irradiation. In addition, anatase TiO_2 is a material well known for its notable photocatalytic properties.[15] This means that the amount of anatase TiO_2 at the TiO_2 surface is one of the critical factors that determine the degree of photocatalytic activity, as indicated previously.[11] According to the TEM and EDX analysis results for the anodized porous TiO_2 implants, amorphous TiO_2 was revealed to be the main constituent of the surface oxide layer of these implants, and anatase TiO_2 formed partially within the amorphous phase. In the current study, once the surface was treated with NH_4F-HF_2 and irradiated with UV light, the modified anodized porous TiO_2 implants showed further improved hydrophilicity. Incidentally, the NH_4F-HF_2-treated, anodized porous TiO_2 implants without UV irradiation and ordinary anodized porous TiO_2 implants showed equivalent wettability (data not shown), indicating that the hydrophilicity obtained in this study was a result of the photocatalytic reaction. Although the surface roughness values differed after the NH_4F-HF_2 treatment, the effect of the surface roughness alteration is thought to be minimal because the surface roughness alteration occurred within the moderately rough surface range, as described by Albrektsson and Wennerberg.[4,5]

The enhanced bone apposition clearly implied improved hydrophilicity. The BMC of TiU was 30.1% at 2 weeks and gradually rose to 37.1% at 6 weeks; however, it was still lower than that of modTiU. Of special note is that the UV-irradiated, NH_4F-HF_2-modified anodized porous TiO_2 implants (modTiU) had induced quick and intensive bone regeneration even at 2 weeks (50.3%), sustaining this high level of activity for the entire 6-week observation period (47.6%). Although the BMC for the modTiU meagerly decreased after 6 weeks compared with 2 weeks, the difference was not significant. This is speculated that the BMC reaches a "plateau" after 2 weeks, enough for firm osseointegration. The corresponding histology showed that the thin but lengthwise new bone contacting the implant at 2 weeks matured and persisted on the implant without major absorption. Because the scattered bone observed at 2 weeks disappeared by 6 weeks, no increase in BMC could be observed. Based on the report by Shimpo and colleagues,[16] the scattered bone probably derived from the periosteal reaction, which in this study was caused during insertion, and the excess bone disappeared over time during remodeling,[17] as seen in week 6. This phenomenon was attributed to the tibia model used in this study. Presumably, if a dog mandible, which has cancellous bone, was used as a model instead, there might have been different results. Anyhow, from a clinical viewpoint, the ability to sustain a high BMC for a long period of time is of great impact. During ordinary osseointegration, the implant stability decreases at some point as bone remodeling proceeds. Most of the implant failures resulting from implant instability occur during this period.[18] It can be speculated that the improved photo-induced hydrophilic surface played a key role in maintaining the high level of stability.

Specifically, the hypothesized association between hydrophilicity and improved bone apposition is mediated by protein adsorption and the subsequent cell response. Protein adsorption on the implant surface begins immediately after insertion, via the patient's blood.[9] One of the major adsorbed proteins is cell-adhesive fibronectin. Serum-derived fibronectin has been reported to enhance osteogenic cell adhesion to the implant surface.[19,20] It has also been reported that higher amounts of fibronectin adsorb onto hydrophilic surfaces than onto hydrophobic surfaces.[21] In the previous study, it was discovered that sufficient amount of fibronectin adsorbed onto the implant surface preferentially enhanced the chemotaxis of osteogenic cells, which leads to faster osseointegration.[22] It was concluded that a surface modification that would induce a higher degree of endogenous fibronectin adsorption would naturally accelerate the osseointegration cascade; this may be one explanation for the enhanced osseointegration seen with the modTiU implants.

The photocatalytic surface has unique properties, among which is the bactericidal effect of the photocatalysts; these photocatalysts decontaminate the surface TiO_2, as reported previously.[23] This opens the door for the decontamination of the implant with surrounding inflammatory tissues and bacteria resulting from peri-implantitis. It has been reported that, on failed implants, foreign elements such as carbon, oxygen, calcium, silicon and aluminum can be detected other than periodontopathic bacteria.[24–27] Furthermore, Buser and colleagues[10] have reported that, even when the implants are installed and kept in glass or plastic ampules, their hydrophilicity decreases because of the exposure to the surrounding air, which contains many foreign elements unfavorable to the success of osseointegration. Intriguingly, the photocatalysts can decompose such foreign elements under UV illumination by generating active oxygen and turn the surface clean.[28]

By using the multiple characteristics of the photocatalysts, a new and comprehensive method for the success of osseointegration solely based on exposure to UV light is proposed. With the photo-reactive surface, preoperative, photo-induced sterilization is possible, keeping the surface as clean as it was at the time of fabrication. In addition, the photo-induced hydrophilic surface enhances early bone apposition, thus shortening the healing period. Finally, the photo-induced decontamination of the infected surface will disinfect periodontopathic bacteria from the implant and render the surface pristine for the achievement of reosseointegration. This advantageous configuration corresponds to the results of the experimental study by Persson and colleagues,[29] who have succeeded in reosseointegrating contaminated sites by substituting the coronal part of a two-piece fixture with a pristine one. They concluded that the quality of the titanium surface is a decisive factor in osseointegration as well as reosseointegration. With the photo-reactive surface, it is unnecessary to change the implant components to decontaminate the area; the infected sites can be decontaminated by simple UV irradiation.

CONCLUSIONS

The NH_4F-HF_2 modification of the anodized porous TiO_2 surface significantly improved its photo-induced hydrophilicity. The photo-induced, hydrophilic, NH_4F-HF_2-modified, anodized porous TiO_2 surface significantly enhanced bone apposition during the early stages of osseointegration.

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